Introduction:

Have you ever thought that colonies of microorganisms taunt our body and have a tremendous impact on how we feel and how we live? And if I tell you that the strength of our bodies fully depends on the trillions of little lives we have inside of us. The human body is home to approximately 100 trillion microorganisms, outnumbering human cells by about 10 to 1. This suggests that the influence of these inhabiting microorganisms is colossal. It is believed that 70%-80% of the human microbiome and 70% of the immune system is located in the gut, since the main task of the intestinal microflora is to protect the body and identify malignant viruses and bacteria. My insatiable interest in researching the connection between autoimmune diseases and the human microbiome is further fueled by the fact that since the early 1980s, the incidence of autoimmune diseases has significantly increased. It is only in recent years that researchers have begun to draw parallels between the causes of these diseases and the imbalance within the human immune system. However, the biggest question of what exactly causes the autoimmune diseases remains unclear in clinical immunology. This intricacy is further exacerbated by the current lack of definitive cures that can combat the disease and its progression. For now, I believe, testing medicines and technologies that can be used to mitigate the disease has a huge cruciality.

This study examines the potential association between changes in the gut microbiome, particularly the prevalence of Prevotella bacteria, and the onset of autoimmune diseases. My research question addresses whether the change in amount of Prevotellas in the human microbiome impacts the development of autoimmune diseases and what kinds of antibiotics can prevent and combat specific types of autoimmune diseases.

I consider the importance of this study to be that today 3-5% of the population suffers from autoimmune diseases, which is about 320,000,000 people, and the very thought that none of them will ever fully recover from the disease is deeply touching.

This research paper will include the research methodologies of testing the efficiency of different antibiotics in the fight against Prevotella bacteria and investigate possible ways of curing autoimmune diseases, thus readers would gain a profound knowledge about how human microbiome (specifically changes in gut microflora) affect the development of autoimmune diseases and

gain a list of the most efficient antibiotics against bacteria that might cause specific types of autoimmune diseases.

Theory part:

An autoimmune illness is a condition caused by the adaptive immune system's abnormal reaction, in which it misidentifies and assaults healthy, functional elements of the body as if they were foreign organisms. The exact reasons and factors that cause autoimmune diseases are still uncertain, they can vary from genetics to environmental and perpetual factors. While the causes of infectious, deficiency, hereditary diseases are fully studied, factors that affect autoimmune diseases and ways of curing them remain unclear.
However, It is obvious that the human microbiome has a huge impact on the progression of autoimmune diseases due to its complexity and relevance in our bodies. Especially gut microflora, as it's populated by trillions of microscopic organisms. These microorganisms consist of more than a thousand types of bacteria, along with viruses, fungi, and parasites. We have symbiotic relations with most of the microorganisms in our gut, as we provide them with shelter and food, they, in fact, hold a significant role in forming the immune system.

Prevotella
Prevotella species are Gram-negative rods most commonly associated with human oral colonization. The human gut is home to several Prevotella species, the most prevalent of which is *Prevotella copri*, which is estimated to have a 40% prevalence in the general human population and relative abundances of over 50% in certain individuals. However, there are conflicting reports that implicate *Previtella copri* in adverse conditions such as rheumatoid arthritis, hypertension and persistent gut inflammation.

Methodology:

The Prevotella bacteria is gram-negative, which makes it more resistant to certain antibiotics and complicates finding the most efficient antibiotical preparation against it. In my research I'm going to be using Prevotella copri, since this species is commonly spread in the gut microbiome. My research methodology is based on Antimicrobial Susceptibility Testing (AST), specifically Disk diffusion method, which is going to help me to understand

the most efficient types of antibiotics in the fight with Prevotella bacterias, thus we can implement them in the autoimmune disease treating protocol. The process of introducing an antimicrobial agent at a certain concentration into a solid culture medium that has been seeded with a chosen inoculum isolated in a pure culture using discs, tablets, or strips is known as disc diffusion (see section 3.i). Based on the identification of an inhibitory zone corresponding to the bacterial susceptibility to the antibiotic contained in the disc, disc diffusion is calculated.
Predicting a bacterial pathogen's potential in vivo response to an antimicrobial treatment is the aim of in-vitro AST. Regardless of whether disc diffusion or dilution procedures are utilized, the results of bacterial in-vitro antimicrobial susceptibility tests are often evaluated and reported as resistant, susceptible, or intermediate to the action of a certain antibiotic. There is no one formula that works for choosing the best breakpoints. The methodology entails an examination of extant data and is subject to the subjectivity of those entrusted with the identification of suitable breakpoints.

The following requirements should be respected:

i) bacteria subjected to AST must be isolated in pure culture from the submitted sample,
ii) standard reference methods should be used for identification so that the subject bacteria are consistently and correctly identified to the genus and/or species level,
iii) bacterial isolates considered to be the most important and a sampling of other isolates, should be stored for future analysis (either lyophilisation or cryogenic preservation at –70°C to –80°C).

Also AST results should be recorded quantitatively:

i) as distribution of MICs in milligrams per liter or μg/ml,
ii) or as inhibition zone diameters in millimeters.

Process:

Step 1. Using an inoculating loop or sterile needle, get samples of Prevotella bacteria and streak onto the upper one-fourth portion of an agar plate with parallel overlapping strokes. Use one agar plate for each testing antibiotic sample. The plate can be divided into half and streaked with two different tissues from the same sample. Be sure to label the plate.

Step 2. Allow the plate to dry for 1-3 minutes. Using sterile forceps or a disk dispenser, place antibiotic disks on the surface of the agar. Ensure the disks are evenly spaced and not too close to the edge of the plate. To guarantee solid contact with the agar, gently press each disc with the forceps.

Step 3. Place all samples into an incubator and set a temperature for 35-36 degrees by cesium. Incubate samples for 16-18 hours.

Step 4. After incubation, examine the plates for clear zones around the disks where bacterial growth has been inhibited. Use a ruler or caliper to measure the diameter of the inhibition zones. Record the measurements in millimeters.

Prevotella species are typically resistant to certain antibiotics, such as penicillin, but they may be susceptible to other ones, in order to find out the most efficient one we have to test multiple types of antibiotics. Based on the AST methodology result we will find out to which of the following antibiotics can target the Prevotella bacterias:

The list of antibiotics:

1. Amoxicillin
2. Ceftazidime
3. Metronidazole
4. Tetracycline

AST Results Table

Antimicrobial agent	MIC/ mg/L			Percentage of isolates with indicated susceptibility		
Killing potential of antibiotics,	range	50%	90%	Susceptible	Intermediate	Resistant

Amoxicillin	<0.016 to >256	6	>256	NA	NA	NA
Ceftazidime	0.094 to >256	6	>256	NA	NA	NA
Metronidazole	<0.016 to >256	0.125	0.38	96%	0	4%
Tetracycline	<0.016 to >256	12	128	36%	15%	49%

Antimicrobial agent		MIC/ mg/L	
Killing potential of an agent, %	range	50%	90%
Amoxicillin	<0.016 to >256	6	>256
Ceftazidime	0.094 to >256	6	>256
Metronidazole	<0.016 to >256	0.125	0.38
Tetracycline	<0.016 to >256	12	128
Piperacillin	<0.016-4	0.023	1,5

Antimicrobial agent		Percentage of isolates with indicated susceptibility	
	Susceptible	Intermediate	Resistant
Amoxicillin	NA	NA	NA
Ceftazidime	NA	NA	NA
Metronidazole	96%	0%	4%

Antimicrobial agent		Percentage of isolates with indicated susceptibility	
Tetracycline	36%	0%	49%
Piperacillin	100%	0%	0%

The following table describes antimicrobial susceptibilities of clinical *Prevotella* isolates. It shows the effectiveness of four antimicrobial isolates that can potentially respond to Prevotella bacteria.
According to the gathered data *Metronidazole* has the highest efficiency:
96% of isolates are susceptible to this antimicrobial agent and only 4% of them are resistant.
The 50% MIC value is 0.125 which is very low. It indicates high efficiency even in low concentrations of Metronidazole. The 90% MIC value is also very low,

Tetracycline has a medium efficiency, and might not be the best option to address Prevotella isolates:
With the susceptibility rate of only 36%, 49% of isolates remain resistant to this antimicrobial agent.
Also the 50% MIC value equals 12, suggesting that Tetracycline is much less potent.

By analyzing the effect of *Piperacillin* we can assume that it has the highest killing capacity:
100% of Prevotella bacterias are susceptible to this antibiotic and the 50% MIC value is 0.023.

Killing capacity of *Amoxicillin* and *Ceftazidime* is very low:
According to the table these antimicrobial agents do not affect Prevotella bacterias.
The 90% MIC value of both antibiotics is >256 which is very high for destroying bacterias. It is less likely to address Prevotellas.

Table results show that Metronidazole is the most efficient antibiotical agent to address Prevotella species.

Discussion

By analyzing the AST (Antimicrobial Susceptibility Test) results we can come to the conclusion that both Metronidazole and Piperacillin have high efficiency in destroying Prevotella species. However, based on the test results, it can be revealed that Piperacillin destroys all bacteria (100%). It may seem at first glance that this is the most suitable option, but do not forget that there are more than a trillion bacteria in the human microbiome and each of them plays a specific and important role in the formation of the immune system. Prevotella species in moderation are important for our body as well and completely destroying them can be dangerous for us. If we look at the table, we will notice that metronidazole leaves a chance for survival for a small number of bacteria species, thereby maintaining the balance of the gut microflora. The 50% MIC value of Metronidazole is 0.125 which in comparison to 50% MIC value of Piperacillin (0.023) is much higher and it might be used in some cases when our end goal is to destroy all bacterias. However, from the medical perspective it is going to be more conscious to use Metronidazole for maintaining a balanced gut microbiome (96% of Prevotella species are susceptible to this microbiological agent and 4% are resistant) since it is part of the normal microflora of the oral cavity, upper respiratory tract, women's reproductive system and a number of other human organs.
Regarding laboratory work, a number of problems arose related to the complexity of the research topic itself. Since I worked mediocrely with bacteria, it was difficult to gain access to the laboratory. I also encountered several difficulties in obtaining the bacteria themselves directly.

Conclusion

In conclusion, the aim of this research paper has been fulfilled. The paper studied the research methodologies of testing the efficiency of different antibiotics in the fight against Prevotella bacteria and investigated possible

ways of curing autoimmune diseases, thus readers gained a profound knowledge about how human microbiome (specifically changes in gut microflora) affect the development of autoimmune diseases and gained a list of the most efficient antibiotics against bacteria that might cause specific types of autoimmune diseases. The hypothesis that there is a link between the changing amount of Prevotella bacterias in our microbiome and the development of autoimmune diseases is approved. Also, the paper identified the best antimicrobial agent to address these bacterias.

References

Băncescu, A., Băncescu, G., Didilescu, A., & Hîrjău, M. (2017). Antibiotic susceptibility testing of some Prevotella strains isolated from vestibular abscesses. FARMACIA, 65(1), 132–133.

Cleveland Clinic. (2024, May 21). Autoimmune diseases. *https://my.clevelandclinic.org/health/diseases/21624-autoimmune-diseases*

OIE Terrestrial Manual 2012. (2012). In OIE Terrestrial Manual. *https://www.woah.org/app/uploads/2015/02/guide-2-1-antimicrobial.pdf*

Ruangpan, L., & Tendencia, E. A. (2004). Laboratory manual of standardized methods for antimicrobial sensitivity tests for bacteria isolated from aquatic animals and environment.

Sherrard, L. J., Graham, K. A., McGrath, S. J., McIlreavey, L., Hatch, J., Muhlebach, M. S., Wolfgang, M. C., Gilpin, D. F., Elborn, J. S., Schneiders, T., & Tunney, M. M. (2013). Antibiotic resistance in Prevotella species isolated from patients with cystic fibrosis. Journal of Antimicrobial Chemotherapy, 68(10), 2369–2374. *https://doi.org/10.1093/jac/dkt191*

Title page

Title: Do the changes in human microbiome affect the development of autoimmune diseases and can the study of these changes fundamentally alter treatment protocols and approaches to autoimmune diseases?

Name: Amina Smak

Aim of the research: Study how the changes in human microbiome affect the trajectory of diseases and conduct the laboratory work to find the best approach to autoimmune disease

Hypothesis: Many autoimmune diseases including, rheumatoid arthritis, systemic lupus erythematosus (SLE) disorders, multiple sclerosis (MS), and juvenile idiopathic arthritis are thought to be brought on by disruption in healthy gut microbiome, or gut dysbiosis.

Objectives: 1) Study the link between microbiome dysbiosis and autoimmune diseases.
2) Based on data gained through the laboratory work, find the best antibiotic against bacteria that might be a cause of some autoimmune diseases.